Acta Alimentaria, Vol. 29 (1), pp. 1–7 (2000)

IN VITRO ANTIOXIDANT EVALUATION OF DANDELION (*TARAXACUM OFFICINALE* WEB.) WATER EXTRACTS

K. HAGYMÁSI[a,d], A. BLÁZOVICS[a], A. LUGASI[b], SZ. T. KRISTÓ[c], J. FEHÉR[a] and Á. KÉRY[c]

[a] II. Department of Medicine, Semmelweis University Medical School, H–1088 Budapest, Szentkirályi u. 46. Hungary
[b] Fodor József National Center of Public Health, National Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/A. Hungary
[c] Institute of Pharmacognosy, Semmelweis University Medical School, H–1085 Budapest, Üllői út 26. Hungary

(Received: 10 May 1999; accepted: 9 September 1999)

Dandelion is a quite widespread medicinal plant, which is widely used as soup, salad, coffee substitute, wine and natural source of flavouring. Its choleretic, diuretic, anti-inflammatory, appetite-stimulating and laxative properties are well known. The aim of this study was to verify the antioxidant properties of lyophilized extracts derived from dandelion root and leaves. The total polyphenol, flavonoid and free SH-group contents of root and leaf extracts were determined spectrophotometrically as well as the hydrogen donating ability and reducing power property. Radical scavenging capacity of extracts was measured in H_2O_2/·OH-luminol-microperoxidase system by chemiluminometric method. The folium extract with approximately 3 times higher polyphenol (9.9 g%) and 6 times higher flavonoid content (0.086 g%) proved to be more effective as hydrogen-donor (I_{50}=160 µg), reducing agent (740 ASE mg^{-1}) and H_2O_2 scavenger (I_{50}=155 µg) compared to radix extract with lower polyphenol and flavonoid content.

Keywords: *Taraxacum officinale* Web., dandelion, antioxidant, lipid peroxidation

Taraxacum officinale Web. (Asteraceae/Compositae) is a member of the daisy family. It is a common plant in temperate climates, particularly in Western Europe, where it inhabits fields, roadsides and waste grounds (LOWELL & ROWAN, 1991). The drug is collected from both wild and cultivated plants. The main suppliers are Bulgaria, former Yugoslavia, Romania, Hungary and Poland (BISSET et al., 1994). The roots are roasted and used as a coffee substitute because of its bitter taste resembling chicory.

[d] To whom the correspondence should be addressed: II. Department of Medicine, Semmelweis University Medical School, H–1088 Budapest, Szentkirályi u. 46. Hungary, E-mail:hkriszti@bel2.sote.hu

0139-3006/2000/$ 5.00

The freshly collected leaves are much appreciated as a salad and soup in the Latin countries. Dried dandelion leaves and roots are available as herbal teas and the powdered root is sold in capsulated form (WILLIAMS et al., 1996). Flowers are used in home wine making (LEUNG & FOSTER, 1996). Dandelion is listed by the Council of Europe as a natural source of food flavouring, especially in alcoholic (e.g. bitters) and nonalcoholic beverages, frozen dairy desserts, candy, baked goods, gelatines, puddings and cheese as well (LEUNG & FOSTER, 1996, NEWALL et al., 1996). Dandelion comes under category N2, which category indicates that dandelion can be added to foodstuffs in small quantities, with a possible limitation of an active principle (as yet unspecified) in the final product (NEWALL et al., 1996). Highest average maximum use levels reported are about 0.014% (143 ppm) for the fluid extract in cheese and 0.003% (33.3 ppm) for the solid extract in baked goods (LEUNG & FOSTER, 1996).

The main constituents of dandelion are: sesquiterpene lactones (germacranolides, eudesmanolides), triterpens including pentacyclic alcohols (taraxol, taraxerol, β-amirin, taraxasterol, arnidiol, farnidiol), phytosterols (sitosterol, stigmasterol), flavonoids (apigenin, luteolin 7-glycosides), acids (caffeic acids, *p*-hydroxyphenylacetic acid, chlorogenic acid, linoleic acid, oleic acid etc.), sugars (fructose, glucose, sucrose), choline, inulin, pectin. The high potassium, calcium and vitamin (A, B, C, D) contents are also worth mentioning.

Dandelions have long been used in herbal medicine for their choleretic, diuretic, antirheumatic, anti-inflammatory, laxative, appetite-stimulating properties for treating liver and gallbladder disorders, digestive complaints (lack of appetite, feeling of distension or flatulence), arthritic and rheumatic diseases as well as eczema and other skin conditions. Frequent contact with dandelions, especially with the latex, may occasionally give rise to contact dermatitis due to taraxacic acid glucoside. Discomfort due to gastric hyperacidity caused by bitter substances may occur with dandelion (BISSET et al., 1994; NEWALL et al., 1996).

Flavonoids can inhibit the activity of enzymes such as lipoxygenase, cyclooxygenase, xantin oxidase, phospholypase A2, protein kinases (CAO et al., 1997; HOLLMAN & KATAN, 1998). Polyphenols in general can influence the lipid peroxidation process in the same way, and can also act as direct scavenger molecules, therefore it can be supposed that they have beneficial, multifactorial effect on the liver microsomal membranes.

Our earlier results demonstrated dandelion's beneficial effect on lipid peroxidation in liver microsomes, in addition its natural extracts were able to stimulate NADPH cytochrome P450 reductase activity (HAGYMÁSI et al., 1999).

Therefore free radical scavenging and/or antioxidant activities of dandelion lyophilized extracts derived from leaves and root were estimated with the determination of hydrogen donating ability, reducing power property and scavenger capacity.

1. Materials and methods

1,1-Diphenyl-2-picrylhydrazyl (DPPH) stable radical, microperoxidase, 5-amino-2,3-dihydro-1,4-phtalazinedion (luminol), cytochrome c and NADPH were obtained from Sigma (St. Luis), glucose-6-phosphate dehydrogenase, serum bovine albumin from Calbiochem AG (Lucerne). All other reagents were purchased from Reanal (Budapest).

The plant material (*Taraxacum officinale* Web. (Asteraceae/Compositae) was collected in 1997. The crude drugs: *Taraxaci folium* and *Taraxaci radix* are commercially available in Hungary, and officially controlled (HUNGARIAN STANDARD, 1970; 1988). Parameters of lyophilization of dry raw materials: LABOR-MIM laboratory instrument, shelf temperature: 60 °C, sample temperature: –20 °C→30 °C, drying time: 13 h, pressure: 12–13 Pa. The lyophilizates were stored at –80 °C and were solved in distilled water just before in vitro examinations.

The polyphenol contents of the lyophilized samples were measured according to the Folin-Denis method spectrophotometrically at 760 nm, using tannic acid as standard (A.O.A.C., 1990).

The flavonoid contents of freeze-dried extracts were determined according to DAB 10 (1991) with aluminium trichloride reagent, after hydrolysis, by spectrophotometric method at 420 nm, and were calculated in hyperoside. With this method the glycosides and aglycons were determined together in aglycon form.

Free SH-groups were determined by the SEDLAK and LINDSAY (1968) method based on ELLMAN (1959) reaction, with the detection of 2-nitro-5-mercapto-benzoic acid at 440 nm.

The hydrogen donating ability of lyophilized extracts from root and leaves was quantified in the presence of 1,1-diphenyl-picrylhydrazyl stable radical on the basis of the BLOIS (1958) method, modified by HATANO and co-workers (1988), at 517 nm spectrophotometrically. Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (CAO et al., 1997). The degradation of DPPH was evaluated by comparison with a control sample, which did not contain hydrogen donating compounds. The amount of the samples (μg) reducing the absorbance by 50% was determined (I_{50}).

The reducing power of lyophilized plant extracts was measured according to the spectrophotometric method of OYAIZU (1986) at 700 nm.

Natural scavenging capacity of lyopohilized samples was detected by chemiluminometric method with Lumat LB 9051 luminometer, according to the method of BLÁZOVICS and co-workers (1999). Unstable free radicals, originated from H_2O_2 in luminol-microperoxidase system via Fenton type reaction, catalysed the transformation of luminol into amino-phtalic acid, when monochromatic light is emitted (expressed RLU: Relative Light Unit). In the presence of free radical scavenging molecules or

compounds the emitted light is reduced. The I_{50} was determined and expressed as μg, that is the amount of the lyphilizate, which diminished emitted light of H_2O_2/·OH-luminol-microperoxidase by 50%.

Results were assessed by one-way analysis of variance (ANOVA) and represent mean ±SEM of three different measurements with 2 parallels.

2. Results

Polyphenol (PF) and flavonoid (F) contents were higher in folium lyophilized extract (PF: 9.9±0.28 g%, F: 0.086±0.003 g%) compared with radix extract (PF: 3.0±0.18 g%, F: 0.014±0.001 g%).

Free SH group contents measured in 1.00 mg ml^{-1} endconcentration of the extracts of this medicinal plant were very low as well (folium: 0.054±0.003 mmol l^{-1}, radix: 0.139±0.006 mmol l^{-1}).

Lyophilized extracts from both parts of dandelion exerted hydrogen donating ability in the presence of DPPH stable radical. The inhibition of the samples showed concentration dependence, as seen in Table 1. The extract from the folium proved to be approximately four times more effective (I_{50} = 160 μg) than the extract from the root (I_{50} = 750 μg).

The samples exhibited reducing power property, which was measured on the basis of Fe(III) to Fe(II) redox reaction. The reducing power property of extracts derived from root and folium showed concentration dependence, as shown in Table 2. The augmented absorbance of the samples showed increased reducing power, which was expressed in ASE mg^{-1}. ASE means that reducing power of 1 mg sample is equivalent to the reducing power of 1 nmol ascorbic acid. The reducing power of folium proved to be 740 ASE mg^{-1} compared with 212 ASE mg^{-1} for that of the radix.

Table 1

Hydrogen donating ability of dandelion folium and root

Absorption (517 nm)		Absorption (517 nm)	
Control	0.521±0.006 A	Control	0.520±0.004A
Taraxaci folium (0.1 mg ml^{-1})		*Taraxaci radix* (1.0 mg ml^{-1})	
200 μl	0.502±0.009 A	200 μl	0.467±0.005 B
500 μl	0.457±0.004 B	500 μl	0.327±0.021 C
750 μl	0.419±0.003 C	750 μl	0.259±0.008 D
1000 μl	0.361±0.010 D	1000 μl	0.177±0.015 E

Data indicated with different capital letters are significantly different ($P<0.05$)

Table 2

Reducing power property of dandelion folium and root

Absorption (700 nm)		Absorption (700 nm)	
Taraxaci folium (1.0 mg ml^{-1})		*Taraxaci radix* (1.0 mg ml^{-1})	
100 µl	0.080±0.003 A	100 µl	0.023±0.000 A
200 µl	0.171±0.002 B	200 µl	0.047±0.000 B
500 µl	0.387±0.007 C	500 µl	0.104±0.001 C
1000 µl	0.696±0.012 D	1000 µl	0.199±0.004 D

Data indicated with different capital letters are significantly different ($P<0.05$)

The H_2O_2 scavenging activity of samples was determined by chemiluminometric method in H_2O_2/·OH-microperoxidase-luminol system. The emitted light of this system was diminished by the extracts in a concentration dependent manner, as it can be seen in Fig. 1. After log/lin transformation linearity could be observed (r^2 folium = 0.9823, resp. radix 0.9907). The extract from folium (I_{50} = 155 µg) proved to be more effective in this system compared with root (I_{50} = 210 µg).

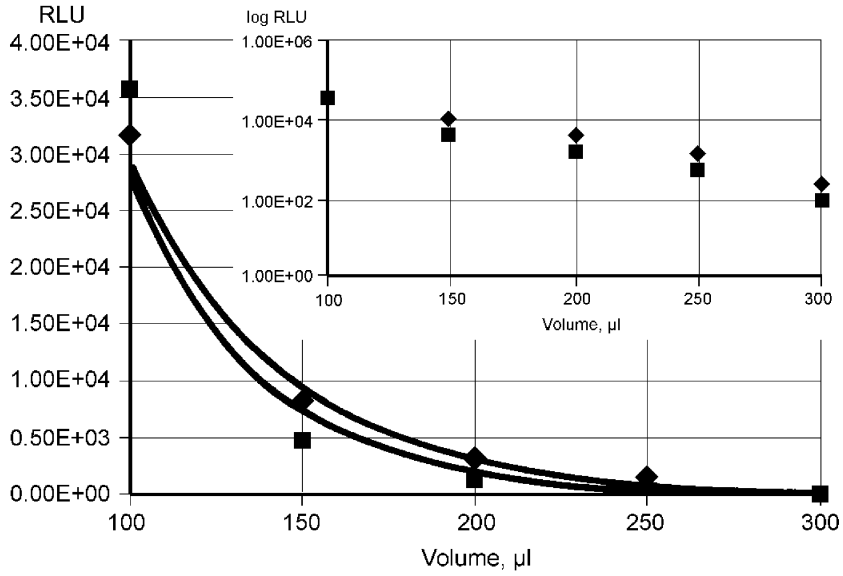

Fig. 1. Total scavenger capacity of natural dandelion extracts in lin/lin and log/lin scale diagram. ♦: *Taraxaci radix* (10 mg ml^{-1}); ■: *Taraxaci folium* (5 mg ml^{-1})

3. Conclusions

There is evidence that free radical reactions can directly or indirectly play major role in cellular processes implicated in many disorders such as atherosclerosis, liver and gallstone diseases, carcinogenesis and so on (HERCBERG et al., 1998). JACOB and BURRI (1996) also suggested that protection against oxidative damage and related diseases is best served by the variety of antioxidant substances found in fruit and vegetables. It is well known from the literature that the concerted use of the antioxidants seems to be more effective than the separate intake (LEE, 1999). A single antioxidant vitamin given high doses to subjects with high risk of pathologies (smokers, asbestos-expose) may not have substantial benefit and could even have negative consequences (HERCBERG et al., 1998). DWORSCHÁK and co-workers (1999) suggested that in the living organism all antioxidant vitamins make a united system with one function lying on the other. This system provides the best way by the intake of natural form as consuming more fruits, vegetables, medicinal plants. The protective role of vegetables, fruit and tea is to provide antioxidant vitamins and specific polyphenols that display a powerful inhibition in oxidative reactions (WEISBURGER, 1998).

Dandelion is well known in herbal medicine for ages. It has a beneficial effect on the treatment of liver, gallbladder diseases, digestive, joint complaints and on the prevention of gallstone formation (BISSET et al., 1994; NEWALL et al., 1996).

Our earlier work (HAGYMÁSI et al., 1999) proved the membrane protecting effect of extracts of this medicinal plant on enzimatically induced and Fe^{3+} stimulated lipid peroxidation in liver microsomes. The compounds, which might act as scavengers of oxigen-reactive derivates, could inhibit microsomal peroxidation and enzyme destruction (VALENZUELA & GUERRA, 1986; NIZAMUDDIN, 1987).

The extracts of folium and root showed H-donating ability, reducing power property and natural scavenging activity in H_2O_2/ ·OH-luminol-microperoxidase system, depending on the concentration. The folium extract with higher polyphenol and flavonoid content proved to be more effective in all three in vitro systems.

Therefore active compounds of dandelion with primary and secondary antioxidant as well as scavenging properties can preserve the structure of membranes and protect against secondary lipid peroxidation, but further in vivo investigations are needed to confirm its existence in human nutrition as a natural source of antioxidants.

*

Authors wish to thank Ms S. BÁRKOVITS, Ms A. KAJÁN and Ms L. TÓTH for their excellent technical assistance.

The research was supported by the Ministry of Welfare, Hungary (ETT 02-517).

References

A.O.A.C. (1990).: Official Methods of Analysis: Tannin in distilled liquors. Arlington, USA, pp. 703–704.

BISSET, N. G., PHILLIPSON, J. D., CZYGAN, F. C., FROHNE, D., HÖLTZEL, D., NAGELL, A., PFANDER, H. J., WILLUHN, G. & BUFF, W. (Eds.) (1994): *Taraxaci radix* cum herba. -in: *Herbal drugs and phytopharmaceuticals*. CRC Press Boca Raton, Ann Arbor, London, Tokyo, pp. 486–489.

BLÁZOVICS, A., KOVÁCS, Á., LUGASI, A., HAGYMÁSI, K., BÍRÓ, L. & FEHÉR, J. (1999): Antioxidant defence in erythrocytes and plasma of patients with active and quescient Crohn's diseases and ulcerative colitis. *Clin. Chem.*, *45*, 895–896.

BLOIS, M. S. (1958): Antioxidant determination by the use of a stable free radical. *Nature*, *181*, 1199–1200.

CAO, G., SOFIC, E. & PRIOR, R. L. (1997): Antioxidant and prooxidant behavior of flavonoids: structure–activity relationship. *Free Rad. Biol. Med.*, *22*, 749–760.

DAB 10 (1991): Deutscher Arzneibuch, 10. Ausgabe. Deutscher Apotheker Verlag, Stuttgart. Gov-Verlag GmbH, Frankfurt/M.

DWORSCHÁK, E., LUGASI, A. & BLÁZOVICS, A. (1999): Antioxidáns vitaminok egészségvédő hatása az újabb emberi megfigyelések tükrében. (The health-protective effect of antioxidant vitamins from the results of nutrition surveys.) *Fitoterápia*, *4*, 3–6.

ELLMAN, G. L. (1959): Tissue sulfhydril group. *Arch. Biochem. Biophys.*, *82*, 70–76.

HAGYMÁSI, K., BLÁZOVICS, A., LUGASI, A., KRISTÓ, T. SZ., FEHÉR, J. & KÉRY, Á. (1999): The in vitro effect of dandelion's antioxidants on microsomal lipid peroxidation. *Phytother. Res.*, in press.

HATANO, T., KAGAWA, H., YASUHARA, T. & OKUDA, T. (1988): Two new flavonoids and other constituents in chicory root: Their relative astringency and radical scavenging effects. *Chem. pharm. Bull.*, *36*, 2090–2097.

HERCBERG, S., GALAN, P., PREZIOSI, P., ALFAREZ, M. J. & VAZQUEZ, C. (1998): The potential role of antioxidant vitamins in preventing cardiovascular diseases and cancer. *Nutrition*, *14*, 513–520.

HOLLMAN, P. C. & KATAN, M. B. (1998): Bioavailability and health effects of dietary flavonols in man. *Arch. Toxicol Suppl.*, *20*, 237–248.

HUNGARIAN STANDARD (1970): *Taraxaci folium*. (*Taraxaci folium*.) MSz 17050–1970.

HUNGARIAN STANDARD (1988): *Taraxaci radix*. (*Taraxaci radix*.) MSz 17658–1988.

JACOB, R. A. & BURRI, B. J. (1996): Oxidative damage and defense. *Am. J. clin. Nutr.*, *63*, 985S–990S.

LEE, I. M. (1999): Antioxidant vitamins in the prevention of cancer. *Proc. Assoc. Am. Physicians*, *111*, 10–15.

LEUNG, A. Y. & FOSTER, S. (1996): Dandelion root. -in: *Encyclopedia of common natural ingredients used in food, drugs, and cosmetics* (2nd. ed.). John Wiley & Sons, New York, pp. 205–207.

LOWELL, C. R. & ROWAN, M. (1991): Dandelion dermatitis. *Contact Dermatitis*, *25*, 185–188.

NEWALL, C. A., ANDERSON, L. A. & PHILLIPSON, J. D. (Eds.) (1996): Dandelion. -in: *Herbal medicines*. The Pharmaceutical Press, London, pp. 96–97.

NIZAMUDDIN, A. (1987): NADPH-dependent and $O^{2\cdot}$-dependent lipid peroxidation. *Biochem. Educ.*, *15*, 58–63.

OYAIZU, M. (1986): Studies on products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.*, *44*, 307–315.

SEDLAK, J. & LINDSAY, R. H. (1968): Estimation of total, protein bound and non protein sulfhydril groups in tissues with Ellmann's reagent. *Anal. Biochem.*, *25*, 192–205.

VALENZUELA, A. & GUERRA, R. (1986): Differential effect of silybin on the Fe^{2+}-ADP and butyl hydroperoxide-induced microsomal lipid peroxidation. *Experientia*, *42*, 139–141.

WEISBURGER, J. H. (1998): Evaluation of the evidence on the role of tomato products in disease prevention. *Proc. Soc. Exp. Biol. Med.*, *218*, 140–143.

WILLIAMS, C. A., GOLDSTONE, F. & GREENHAM, J. (1996): Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of *Taraxacum officinale*. *Phytochemistry*, *42*, 121–127.